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Differential Regulation of Slow and Rapid Delayed Rectifier Potassium Currents by cGMP Dependent Nitric Oxide Signalling Pathways in Isolated Adult Guinea Pig Ventricular Myocytes

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Nitric oxide (NO) signalling pathways are reported to regulate cardiac repolarisation. This was investigated using BAY 60-2770, which activates soluble guanylyl cyclase (sGC) in a NO and haem independent manner to generate cGMP. Action potentials (APs) and currents were recorded from isolated guinea pig ventricular myocytes at 37°C using the perforated patch clamp technique. APs were stimulated at 2 Hz. The slow and rapid delayed rectifier potassium currents, I_{Ks} and I_{Kr} were measured as tail currents elicited by a voltage step from +40 mV to −50 mV. The following experiments were carried out in the presence of 100 μM IBMX (3-Isobutyl-1-methylxanthine) a non-selective phosphodiesterase (PDE) inhibitor. 1 μM BAY 60-2770 lengthened APD_{90} by 15.7 ms ($p<0.05$, n=7), suggesting that sGC activation and an increase in cGMP inhibits repolarising currents. Further experiments showed that I_{Ks} was inhibited by BAY 60-2770. IBMX alone enhanced I_{Ks} by 77 ± 9 % ($p<0.001$, n=8) and subsequent addition of BAY 60-2770 reduced the IBMX dependent enhancement of I_{Ks} by 68 ± 2 % ($p<0.001$, n=8). This suggests that PDE inhibition permits I_{Ks} to be inhibited by cGMP dependent NO signalling pathways. The sGC mediated inhibition of I_{Ks} by BAY 60-2770 is unlikely to be due to phosphorylation by protein kinase G (PKG) because the inhibition remained in the presence of 100 nM KT-5823, a PKG inhibitor and because PKG activation with 100 μM 8-bromo-cGMP had no effect on I_{Ks}. I_{Kr} was not regulated by PDE inhibition, sGC activation or PKG activation. Overall, these findings suggest that cGMP dependent NO signalling pathways regulate I_{Ks}, but not I_{Kr}, via a PKG independent mechanism.

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A Trek-Like K^+ Channel Current Inhibited by Norepinephrine in Rat Atrial Myocytes

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Accumulating evidence indicates that two-pore domain K^+ (K_{2P}) channels contribute to atrial background K^+ currents. Atrial background K^+ currents are modulated by norepinephrine (NE). However, the identity of the underlying channels and the mechanisms of their modulation remain unclear. This study aimed to investigate the norepinephrinergic modulation of rat atrial K^+ currents. Whole-cell rat atrial myocyte currents were recorded at 36°C. NE (1 μM) increased L-type Ca^{2+} current (I_{CaL}) by 205 ± 40 % (n=6) and inhibited the steady-state outward current (I_{Kss}) by 42.1 ± 4.3 % (n=7). The NE-dependent inhibition of I_{Kss} was: abolished by blockers of the K_{2P} channel, TREK-1 (K_{2P}2.1), fluoxetine (100 μM) and mibefradil (2.5 μM), partially reduced by 4-aminopyridine (3 mM, 14.6 ± 2.7 %, n=5; $P<0.0001$) and unaffected by blockers of the K_{2P} channels, TASK-1 (K_{2P}3.1) and TASK-3 (K_{2P}3.3), Zn^{2+} (1 mM). Conversely, NE-dependent inhibition of I_{Kss} was potentiated (60.9 ± 3.9 %, n=5; $P<0.01$) by the arachidonic acid analogue, EYTA (10 μM). Noise analysis revealed a unitary conductance of 33.0 ± 7.5 pS for the NE-sensitive channel (n=8). Theeffectof 1 μM NE on I_{Kss} was abolished by β_1/β_2-adrenoceptor non-selective propranolol (1 μM). The action of NE on I_{Kss}, but not I_{CaL}, was abolished by pertussis toxin-treatment. NE prolonged APD_{30} by 52 ± 19 % (n=5; $P<0.05$). In conclusion, NE inhibits a TREK-like K^+ channel current via a pertussis toxin-sensitive pathway that contributes to action potential prolongation in rat atrial myocytes.

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Modulation of K_{2P} K^+ Leak Channel Sensitivity to Carvedilol by Alternative MRNA Translation Initiation

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The β-receptor antagonist carvedilol exerts multichannel blocking properties. K_{2P}2.1 (TREK1) and K_{2P}10.1 (TREK2) channels are expressed in the heart and regulated by alternative translation initiation (ATI) of their mRNA, producing functionally distinct channel variants. The first objective was to investigate acute effects of carvedilol on human K_{2P}2.1 and K_{2P}10.1 channels. Second, we sought to study ATI-dependent modulation of K_{2P} K^+ current sensitivity to carvedilol.

Wild type and mutant K_{2P}2.1 and K_{2P}10.1 currents were recorded from *Xenopus* oocytes and HEK 293 cells. Carvedilol caused concentration-dependent inhibition of K_{2P}2.1 channels ($IC_{50,oocytes}$ = 20.3 μM; $IC_{50,HEK}$ = 1.6 μM). The drug targeted K_{2P}2.1 channels in voltage-dependent and frequency-independent fashion. When K_{2P}2.1 isoforms generated by ATI were studied in isolation in oocytes, the IC_{50} value for carvedilol inhibition of full-length channels (16.5 μM) differed by 4.8-fold from the truncated channel variant (IC_{50} = 79.0 μM). Similarly, related K_{2P}10.1 channels were blocked by carvedilol ($IC_{50,oocytes}$ = 24.0 μM; $IC_{50,HEK}$ = 7.6 μM) and subject to ATI-dependent modulation of drug sensitivity.
In conclusion, carvedilol targets K_{2P}2.1 and K_{2P}10.1 K^+ channels. Drug sensitivity of cardiac ion channels K_{2P}2.1 and K_{2P}10.1 is modulated by alternative mRNA translation initiation.

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L-Type Calcium and Potassium Currents are Differently Regulated by Angiotensin II in Atrial and Ventricular Mouse Myocytes

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Atrial fibrillation (AF) is the most commonly encountered arrhythmia in clinical practice. Additionally, cardiac hypertrophy and arrhythmias have been associated with chronic activation of the renin-angiotensin system (RAS) during which the detrimental effects of angiotensin II (ANG II) are mediated by the type 1 ANGII receptor (AT1R). Nonetheless, it remains poorly understood whether a cause-effect relation exist between AT1R overstimulation and AF. Previously, we demonstrated that mice with cardiac-specific overexpression of AT1R (AT1R mice) had reduced ventricular K^+ and L-type Ca^{2+} currents (I_{CaL}). Conceivably, similar changes in these ionic currents could occur in the atria thus, promoting the development AF. Accordingly, we hypothesize that AT1R mice have reduced density of K^+ currents and I_{CaL}.
Atrial myocytes were isolated from control and AT1R male mice at age 50 and 180 days specifically, before and after the occurrence of structural cardiac remodelling. Ionic current density (pA/pF) was measured by the patch-clamp technique. Data shows that at 180-days, cell capacitances were 43% higher in AT1R mice compared to controls, but total K^+ currents (at +30 mV) were similar between control (14.1 ± 0.9, n=39) and AT1R (12.6 ± 0.8, n=31). Moreover, peak I_{CaL} densities in 50-days were also comparable between these two groups (CTL: −4.2 ± 0.3, n=21; AT1R:-3.7 ± 0.2, n=25). In contrast, at 180-days, a 33% decrease in maximum I_{CaL} was noted in the AT1R mice atrial myocytes compared to controls. (CTL: −5.4 ± 0.4, n=28; AT1R: −3.6 ± 0.2, n=26). This reduction in I_{CaL} density was correlated with a decrease in mRNA expression of the $Ca_V1.2$ underlying subunit.
Overall, this study shows that I_{CaL} but not K^+ currents are modulated by an ANGII-induced atrial hypertrophy, also highlighting a different regulation of these ionic currents in the atria compared to the ventricles.

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Nonlinear Behavior of Conduction in Cardiac Tissue with Heterogeneous Expression of Connexin 43

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Altered cardiac gap junctional coupling is known to potentiate slow conduction and arrhythmias. However, the effects of heterogeneous connexin expression have scarcely been studied. Therefore, our aim was to investigate conduction in tissue consisting of two cardiomyocyte populations expressing different connexin levels.
Conduction velocity was measured using microelectrode arrays in cultured strands of fetal murine ventricular myocytes with predefined contents of connexin 43 knock-out (Cx43KO) cells (0 to 100% in steps of 10%) after 1 min of pacing at 3.33 Hz. Corresponding computer simulations of conduction with a subcellular discretization (2.5 μm) were run in randomly generated two-dimensional tissues mimicking the cellular architecture of the strands.
In the cultures, the relationship between CV and Cx43KO cell content was highly nonlinear. CV first decreased abruptly from 42.1 ± 2.7 to 7.5 ± 2.3 cm/s when Cx43KO content was increased from 0 to 50% (n=52 and 12, respectively, $p<0.001$). When the Cx43KO content was ≥60%, CV became comparable to that observed in 100% Cx43KO strands (2.3 ± 0.2 cm/s, n=18). Furthermore, combining Cx43KO and wild-type cells resulted in heterogeneous conduction patterns. The simulations replicated this behavior of conduction. For Cx43KO contents of 10-50%, conduction was slowed due to wavefront meandering between Cx43KO cells. For Cx43KO contents